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Research on the Influence of Organic Fertilizing and Mulching on Productivity and Phytodiversity on Oligotrophic Grasslands

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Abstract. Organic fertilizers have an influence on the floristic composition of grasslands. Fertilizing with manure favours the phytodiversity of grasslands. The objective of the study was to follow the influence of mulching and organic fertilizing in small quantities and at various time intervals upon dry matter yield and phytodiversity of *Agrostis capillaris* L. - *Festuca rubra* L. grassland type in the Apuseni Mountains. Seven treatments (control (mowed 1/year); mulch 1/year; mulch 1/year + 5 t/ha manure /year; mulch 1/year + 5 t/ha manure/2 years; mulch 1/year + 10 t/ha manure/2 years; mulch 1/year + 10 t/ha manure/3 years; abandonment) were evaluated experimentally. Organic fertilizing in low quantities could be a way to maintain the floristic diversity of oligotrophic grasslands in Apuseni Mountains.

Keywords: management of grasslands, floristic composition, organic fertilization, mulching.

INTRODUCTION

Organic fertilizing, with manure, of the oligotrophic grasslands in Apuseni Mountains, in a traditional way, lead to a specific phytodiversity (Morea, 2008, Păcurar *et al.,* 2011). Fertilization with manure could maintain the phytodiversity of the grasslands (Nettier *et al.*, 2010). Manure is a good fertilizer and is indicated on the permanent grassland, with high diversity (Briemle, 1991). Permanent and semi-natural grasslands offer good conditions for a large diversity of habitats and species (Vidrih *et al.*, 2010). The best way to maintain and increase the areas of semi-natural grasslands is the extensive management. (Köster *et al.*, 2011). Agricultural policy developments and management, farming practices and techniques are needed on grassland and other pastoral ecosystems, that can be implemented to help deliver nature conservation objectives (Hopkins *et al.*, 2006). Nature 2000 and agri-environmental programs should help in grassland protection, but in practice the results are not satisfying, because farmers are still not very interested in environmental protection of their grasslands (Stypiński *et al.*, 2007).

The aim of the study was to find out the influence of mulching and organic fertilizing in small quantities and at various time intervals upon dry matter yield and phytodiversity of *Agrostis capillaris* L. - *Festuca rubra* L. grassland type, in the Apuseni Mountains, to maintain semi-natural oligotrophic grasslands.

MATERIAL AND METHOD

The experiment was carried out in a place called Bear Meadows (1349 m), Ocoale Village, Gârda de Sus Commune. The experimental field was established in 7 variants and 5 replications: V 1 – control (mowed 1/year); V 2 – mulch 1/year; V 3 – mulch 1/year + 5 t/ha manure /year; V 4 – mulch 1/year + 5 t/ha manure/2 years; V 5 - mulch 1/year + 10 t/ha

manure/2 years; V 6 - mulch 1/year + 10 t/ha manure/3 years; V 7 –abandonment. The floristic composition was interpreted by Braun-Blanquet method (Braun-Blanquet, 1932), when the *Poaceae* species were in flower (in July, 2012). For the processing of floristic data, we used the PC-ORD program, which performs the multivariate analysis of the ecological data entered into the spread sheet. This program focuses on nonparametric tools, on graphics, randomization tests, bootstrapped confidence intervals for analysis of community data (McCune *et al*., 2011). For data processing and interpretation we used the MRPP (Multi Response Permutation Procedure), multidimensional scaling NMDS, Summary (for the Shannon index and the number of species) and average abundance and dominance. MRPP compares differences within the group and between groups. The method involves using the T test, which describes the separation between groups. The negative T indicate that the separation is stronger. The P-value is useful to assess if the observed difference is just an incident or not. It was also used a non-metric method, the multidimensional scaling (NMDS), which is well suited to data coordination that are not normal or stairs arbitrary, discontinuous, or otherwise questionable (Peck, 2010). For variance analysis and evaluate the effect of treatments applied on vegetation, we used STATISTICA program, created by the company StatSoft. The analysis of variance was performed by Breakdown and One-Way ANOVA type. Based on F and p values was determined the species to which treatments were performed highest effect, based on the average abundance-dominance of the species. To analyse in detail the effect of treatment on species with significant reaction, we used a comparative analysis, *Post-hoc, the Fisher LSD* type. The choice of this test was based on the ability to highlight significant differences between the experimental variants.

RESULTS AND DISCUSSIONS

The research was performed on an *Agrostis capillaris* L. - *Festuca rubra* L. grassland type. The floristic composition of the experimental variants greatly overlaps, which shows high similarity of the phytocoenoses, indicating that small quantities of organic fertilization combined with mulching, have not caused major changes in the floristic composition (2012) on a this grasslnad (Fig.1).

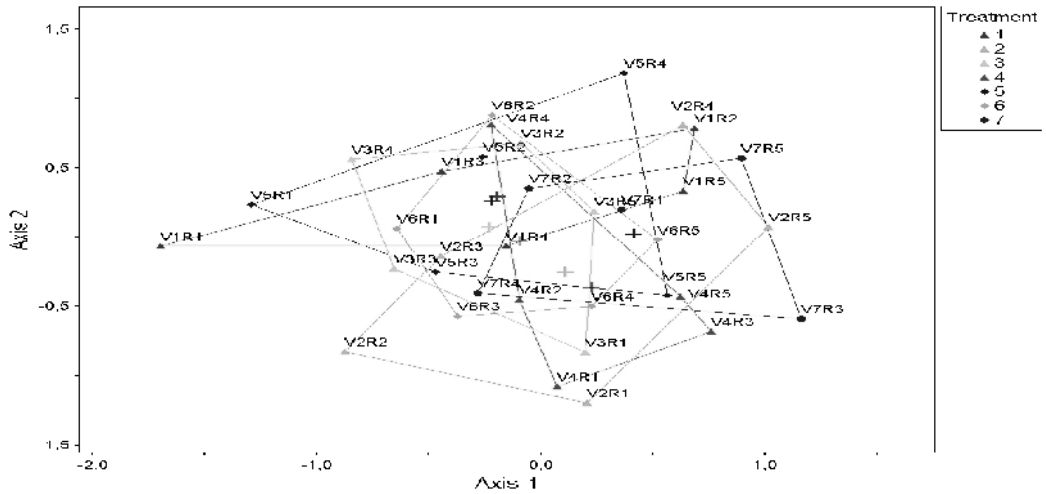

Fig. 1. Ordering floristic composition in 2012, according to combined organic mulching treatments (treatments: V1 - control, V2 mulching/year, V3-mulching/year + 5 t/ha manure, applied annually, V4 - mulching/year + 5 t/ha manure, applied once to 2 years, V5-mulching/year + 10 t/ha manure, applied once to 2 years, V6-mulching/year + 10 t/ha manure, applied once to 3 years, V7-meadow abandoned, R-repetitions)

Comparing the results of each of the variants' floristic composition, it can be seen that there are small changes, but only the treatment mulching/year + 5t/ha manure, applied annually (V3), compared with abandonment (V7), has statistically significant differences (p <0.05, Tab.1). Cristea *et al.,* 2004 affirm that the changes taking place in the structure of phytocoenoses under the action of disturbances, giving to this a certain dynamics. Not all the changes will lead to the destruction or replacement of phytocoenoses, but some parameters may suffer significant changes, within the capacity of self-regulation. Oscillatory dynamic processes are related to internal dynamics, involving quantitative and reversible changes without substituting one type of phytocoenosis with other (Falinski, 1986).

Tab.1

Comparing floristic composition in 2012 experimental variants with MRPP
(T - T test, A - homogeneous group, p - statistical significance)

Comparing variants	T	A	*p*	Significance
V1 vs. V2	-0.40100544	0.015402	0.2978	ns
V1 vs. V3	-0.90723956	0.026093	0.1738	ns
V1 vs. V4	-1.07920779	0.026351	0.1408	ns
V1 vs. V5	0.92903997	-0.030462	0.8381	ns
V1 vs. V6	-0.45061778	0.013052	0.2981	ns
V1 vs. V7	-0.97901303	0.030155	0.1567	ns
V2 vs. V3	-0.71060606	0.025025	0.2213	ns
V2 vs. V4	-0.19796931	0.005759	0.3961	ns
V2 vs. V5	-0.56065414	0.019308	0.2529	ns
V2 vs. V6	-0.36899425	0.011846	0.3324	ns
V2 vs. V7	-1.06939998	0.038349	0.1401	ns
V3 vs. V4	-0.97920457	0.031361	0.1609	ns
V3 vs. V5	0.10191392	-0.002829	0.4939	ns
V3 vs. V6	0.91783515	-0.033814	0.8244	ns
V3 vs. V7	-2.15449180	0.060516	0.0247	*
V4 vs. V5	0.17848870	-0.006848	0.4864	ns
V4 vs. V6	0.34730960	-0.011922	0.6068	ns
V4 vs. V7	0.18526432	-0.005493	0.5323	ns
V5 vs. V6	0.78711614	-0.027245	0.7734	ns
V5 vs. V7	-0.90342233	0.028589	0.1668	ns
V6 vs. V7	-0.42858691	0.013554	0.3041	ns
Note : * = p <0.05. ** = p <0.01. *** = p <0.001. 0 = p <0.05. 00 = p <0.01. 000 = p <0.001. ns - not significant).				

All the changes in the vegetation occurring within *Agrostis capillaris* L. - *Festuca rubra* L. grassland type.

The dominant species *Agrostis capillaris L.* (23.5%) slightly reduced its share under the influence of experimental factors, but without statistically significant differences, reaching 19.5% at the variant mulching/year + 5 t/ha manure, applied once to 2 years (V4), mulching/year + 10 t/ha manure, applied applied once to 3 years (V6), compared to the control (V1). The cover of this species in the variant mulching/year + 10 t/ha of manure, applied once to 2 years (V5) is maintained at the same level as in the control (V1, Tab. 2).

Codominant species *Festuca rubra L.* (12.5%) had increased its share compared to the control (V1) in the following variants: mulching/year (V2), mulching/year + 5 t/ha manure, applied once to 2 years (V4), but without statistically significant differences. There is a decline in the species share in the following situations: mulching/year + 5 t/ha manure,

applied annually (V3), mulching/year + 10 t/ha manure, applied once to 3 years (V6), abandoned grassland (V7), still without statistically significant differences (Tab. 2).

The participation of the *Poaceae* is 37.85% (V1-control), but it changes slightly to 38.5% at the variants mulching/year + 10 t/ha manure, applied once to 2 years (V5). In all of the other cases the share of the *Poaceae* shows an easy decrease, but without statistically significant differences (Tab. 2). The *Fabaceae* are present in 10.5% and shows a decrease in each of the variants compared to the control (V1), with statistically significant differences ($p < 0.05$), in the case of abandoned pastures (V7, Tab. 2). The *Cyperaceae* and *Juncaceae* are represented by 0,4% in the control (V1) and have the aproximately same share in the other experimental variants, without statistically significant differences, which means they are less influenced by the experimental factors.

In the case of the *Plants from other botanical families (ABF)*, the results do not show statistical assurance, but fluctuations can be observed regarding their participation in floristic composition (Tab. 2). Compared with the control (V1, 54.7%) the *ABF* share increase in the next situations: mulching/year (V2), mulching/year + 5 t/ha manure, applied once to 2 years (V4), mulching/year + 10 t/ha manure, applied once to 3 years (V6), abandonment (V7). In the case of mulching/year + 5 t/ha manure, applied annually (V3) and mulching/year + 10 t/ha manure, applied once to 2 years (V5), the presence of *ABF* decrease compared to the withness (V1), but these changes do not have statistical assurance.

Tab. 2

Floristic structure types of grassland in 2012 under the influence of organic inputs combined with mulching (Ac = *Agrostis capillaris* L., Fr =*Festuca rubra* L. P = *Poaceae*, F = *Fabaceae* CJ = *Cyperaceae* and *Juncaceae*, AFB = other botanical families, Ao =*Anthoxanthum odoratum* L. Tp = *Trifolium pratense* L. Ra = *Ranunculus acris* L. Vch = *Veronica chamaedrys* L. T = treatment, *S**. = Meaning, H = Shannon Index, S = number of species, Ac-Fr = *Agrostis capillaris* L. - *Festuca rubra* L. D=Dominant, CD=Codominant, Phytodiv.=Phytodiversity, Typ=Meadow type, Mt. = control, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, 0 = $p < 0.05$, 00 = $p < 0.01$, 000 = $p < 0.001$)

		Sp. D. and CD		Economic groups				Phytodiv.	
	Type	A.c.	F.r.	P	F	CJ	AFB	S	H
V1	A.c-F.r	23.5	12.5	37.85	10.15	0.4	54.7	27,8	2.49
***S**.**		Mt	Mt.	Mt.	Mt.	Mt.	Mt.	Mt.	Mt.
V2	A.c-F.r	19.45	13.75	35.95	5.7	0.4	55.95	26.8	2.46
***S**.**		ns	ns	ns	ns	ns	ns	ns	ns
V3	A.c-F.r	21.5	11.25	35.25	9.5	0.3	48.55	25.8	2.53
***S**.**		ns	ns	ns	ns	ns	ns	ns	ns
V4	A.c-F.r	19.5	13.75	34.65	6.7	0.3	56.10	27.00	2.37
***S**.**		ns	ns	ns	ns	ns	ns	ns	ns
V5	A.c-F.r	23.5	12.5	38.3	9.65	0.2	53.45	27.4	2.46
***S**.**		ns	ns	ns	ns	ns	ns	ns	ns
V6	A.c-F.r	19.5	11.25	32.25	9.5	0.3	57.65	26.8	2.57
***S**.**		ns	ns	ns	ns	ns	ns	ns	ns
V7	A.c-F.r	21.5	10	32.00	3.4	0.1	68.70	27.00	2.44
***S**.**		ns	ns	ns	°	ns	ns	ns	ns

Phytodiversity is reduced from 27.8 species to 25.8 in the variant of mulching/year + 5 t/ha manure, applied annually (V3), but without statistical assurance. Considering Shannon biodiversity index, coluld be observed that in comparison to control (V1) there is a slight increase at the variant of mulching/year + 10 t/ha manure, applied once to 3 years (V6), without statistically significant differences. For other variants (mulching/year (V2),

mulching/year + 5 t/ha manure, applied once to 2 years (V4), mulching/year + 10 t/ha manure, applied once to 2 years (V5), abandoned grassland (V7)) the Shanon index value decreases, but without statistical assurance (Tab. 2). In general the species, does not indicate significant effects of organic fertilizing combined with mulching. The reaction of the species *Agrostis capillaris* L. to the applied treatments, isn't in the statistically significant difference frame ($p>0.05$), in any of the variants. If comparing *Festuca rubra* L. response to withness (V1), there are no notable effects, the results haven't statistical assurance. Only in the abandonment (V7) is significantly lower ($p <0.05$), than in the cases of mulching/year (V2) and mulching/year + 5 t/ha manure, applied once to 2 years (V4, Tab. 3).

Tab. 3

Response of the species *Festuca rubra* L. to the applied treatments (LSD test)

Variants	-	V1	V2	V3	V4	V5	V6	V7
-	ADm	12.50	13.75	11.25	13.75	12.05	11.25	10.00
V1	12.50		0.4514	0.4514	0.4514	1.0000	0.4514	0.1378
V2	13.75			0.1378	1.0000	0.4514	0.1378	0.0297°
V3	11.25				0.1378	0.4514	1.0000	0.4514
V4	13.75					0.4514	0.1378	0.0297°
V5	12.50						0.4514	0.1378
V6	11.25							0.4514
V7	10.00							

Note: $p < 0.05$ * ° $p < 0.01$ ** °° $p < 0.001$ *** °°° ADm – average abundence dominance V1 - control, V2 mulching/year, V3-mulching/year + 5 t/ha manure, applied annually, V4 - mulching/year 5 t/ha manure, applied once to 2 years, V5-mulching/year + 10 t/ha manure, applied once to 2 years, V6-mulching/year + 10 t/ha manure, applied once to 3 years, V7- abandoned meadow

The following species have responded, with statistically significant differences, to the fertilization with small quantities of organic fertilizers combined with mulching: *Antoxanthum odoratum* L., *Trifolium pratense* L., *Ranunculus acris* L., *Veronica chamaedrys* L..

In terms of *Antoxanthum odoratum* L. species abundance, could be observed a negative significant effect of abandomnment (V7), compared to control (V1), with statistical assurance ($p <0.05$). Also there are very significant negative effects of abandonment ($p<0.01$) in comparison with the following treatments: mulching/year (V2), mulching/year + 5 t/ha manure, applied annually (V3), mulching/year + 10 t/ha manure, applied once to 2 years (V5). The variant mulching/year + 5t/ha manure, applied once to 2 years (V4) has significant adverse effects ($p<0.05$) on the species in comparison to mulching once a year (V2). The variant mulching/year + 10 t/ha manure, applied once to 3 years (V6) has significant negative effects ($p<0.05$) on the species comparing to mulching once a year (V2, Tab.4).

In the case of *Trifolium pratense* L., mulching once a year (V2), compared to control (V1) has statistically significant differences ($p <0,05$). Treatment mulching/year + 5 t/ha manure, applied once to 2 years (V4) brings very significant negative differences ($p<0.01$) compared to the control (V1) and significant differences ($p<0.05$) compared to mulching/year + 5 t/ha manure, applied annually (V3). The variant mulching/year + 10 t/ha manure, applied once to 3 years (V6) has positive effects in comparison to the treatment mulching/year + 5 t/ha manure, applied once to 2 years (V4).

The species *Ranunculus acris* L. shows positive significant differences in the case of the variant mulching/year + 5 t/ha manure, applied once to 2 years (V4), compared to the control (V1). Abandonment (V7) has very significant positive effect compared to: control (V1), mulching/year + 5 t/ha manure, applied annually (V3) and mulching/year + 10 t/ha manure, applied once to 2 years (V5).

Tab. 4

Response of the species *Anthoxanthum odoratum* L. to the applied treatments (LSD test)

Variants	-	V1	V2	V3	V4	V5	V6	V7
-	ADm	1.85	2.75	2.30	1.30	2.30	1.40	0.50
V1	1.85		0.1616	0.4781	0.3870	0.4781	0.4781	0.0398°
V2	2.75			0.4781	0.0281°	0.4781	0.0398°	0.0012°°
V3	2.30				0.1214	1.0000	0.1616	0.0076°°
V4	1.30					0.1214	0.8742	0.2117
V5	2.30						0.1616	0.0076°°
V6	1.40							0.1616
V7	0.50							

Note: $p < 0.05$ * ° $p < 0.01$ ** °° $p < 0.001$ *** °°° ADm – average abundence dominance V1 - control, V2 - mulching/year, V3 - mulching/year + 5 t/ha manure, applied annually, V4 - mulching/year 5 t/ha manure, applied once to 2 years, V5 - mulching/year + 10 t/ha manure, applied once to 2 years, V6 - mulching/year + 10 t/ha manure, applied once to 3 years, V7- abandoned meadow

Abandonment (V7) has very significantly affected the reaction of the species *Trifolium pratense* L. compared to the control ($p < 0,01$). In comparison with the following tretments: mulching/year + 5 t/ha manure, applied annually (V3) and mulching/year + 10 t/ha manure, applied once every 3 years (V6), abandonment had statisitically significant negative effects (Tab.5). Significant positive effects can be observed at mulching/year + 5 t/ha manure, applied annually (V3) and mulching/year + 10 t/ha manure, applied once every 3 years (V6).

Tab. 5

Response of the species *Trifolium pratense* L. to the applied treatments (LSD test)

Variants	-	V1	V2	V3	V4	V5	V6	V7
-	ADm	6,25	2,75	5,80	1,85	4,55	5,80	1,85
V1	6.25		0.0280°	0.7679	0.0070°°	0.2699	0.7679	0.0070°°
V2	2.75			0.0531	0.5560	0.2433	0.0531	0.5560
V3	5.80				0.0142°	0.4149	1.0000	0.0142°
V4	1.85					0.0847	0.0142*	1.0000
V5	4.55						0.4149	0.0847
V6	5.80							0.0142°
V7	1.85							

Note: $p < 0.05$ * ° $p < 0.01$ ** °° $p < 0.001$ *** °°° ADm – average abundence dominance V1 - control, V2 - mulching/year, V3 - mulching/year + 5 t/ha manure, applied annually, V4 - mulching/year 5 t/ha manure, applied once to 2 years, V5 - mulching/year + 10 t/ha manure, applied once to 2 years, V6 - mulching/year + 10 t/ha manure, applied once to 3 years, V7 - abandoned meadow

Regarding the species *Veronica chamaedrys* L., the abandonment (V7) has significantly distinct positive effects on species abundance-dominance, in comparison to treatment mulching/year (V2), and significant effects compared to the following: mulching/year + 5 t/ha manure, applied annually (V3), mulching/year + 10 t/ha manure, applied once to 2 years (V5), mulching/year + 10 t/ha manure, applied once every 3 years (V6). The highest yield of dry matter (D.M.), 0,66 t/ha, was found in a variant mulching once a year + 10 t/ha manure, applied once to 2 years (V5), with a statistically significant difference, compared with the control ($p < 0.05$, Tab.8), while the lowest recorded harvest was in variant mulching once a

year (V2) with a difference of -0.11 t/ha compared to the control, but without statistical assurance (p> 0.05).

Tab. 6

Response of the species *Ranunculus acris* L. to the applied treatments (LSD test)

Variants	-	V1	V2	V3	V4	V5	V6	V7
-	ADm	0	0,1	0,2	0,3	0,1	0,2	0,5
V1	0		0.4692	0.1534	0.0361*	0.4692	0.1534	0.0010**
V2	0.1			0.4692	0.1534	1.0000	0.4692	0.0066**
V3	0.2				0.4692	0.4692	1.0000	0.0361*
V4	0.3					0.1534	0.4692	0.1534
V5	0.1						0.4692	0.0066**
V6	0.2							0.0361*
V7	0.5							

Note: p < 0.05 * ° p < 0.01 ** °° p < 0.001 *** °°° ADm – average abundence dominance V1 - control, V2 - mulching/year, V3 - mulching/year + 5 t/ha manure, applied annually, V4 - mulching/year 5 t/ha manure, applied once to 2 years, V5 - mulching /year + 10 t/ha manure, applied once to 2 years, V6 - mulching/year + 10 t/ha manure, applied once to 3 years, V7 - abandoned meadow

Tab. 7

Response of the species *Veronica chamaedrys* L. to the applied treatments (LSD test)

Variants	-	V1	V2	V3	V4	V5	V6	V7
-	ADm	4.35	0,40	3,55	4,35	3,10	3,55	8,30
V1	4.35		0.0618	0.6966	1.0000	0.5431	0.6966	0.0618
V2	0.40			0.1320	0.0618	0.1943	0.1320	0.0006***
V3	3.55				0.6966	0.8262	1.0000	0.0267*
V4	4.35					0.5431	0.6966	0.0618
V5	3.10						0.8262	0.0161*
V6	3.55							0.0267*
V7	8.30							

Note: p < 0.05 * ° p < 0.01 ** °° p < 0.001 *** °°° ADm – average abundence dominance V1 - control, V2 - mulching/year, V3 - mulching/year + 5 t/ha manure, applied annually, V4 - mulching/year 5 t/ha manure, applied once to 2 years, V5 - mulching /year + 10 t/ha manure, applied once to 2 years, V6 - mulching/year + 10 t / ha manure, applied once to 3 years, V7 - abandoned meadow

Tab 8

Influence of organic fertilization on dry matter yield (2012)

Experimental treatments	t/ha SU (DM)	%	Differences	Significance
V1-control	0.47	100.0	0.00	Mt.
V2-mulching/year	0.35	76.0	-0.11	-
V3- mulching/year + 5t/ha manure, aplied annualy	0.56	119.7	0.09	-
V4- mulching/year +5t/ha manure, aplied1x at 2 years	0.48	103.4	0.02	-
V5- mulching/year + 10 t/ha manure, aplied 1x at 2 years	0.66	141.2	0.19	*
V6- mulching/year + 10 t/ha manure, aplied 1x at 3 years	0.49	106.0	0.03	-
Note: DL (p 5%) - 0.18; DL (p 1%) - 0.24; DL (p 0.1%) - 0.33				

The results of our experience on organic fertilization on grassland production does not fall into the pattern of other experiences with organic fertilizer, but put it to the fact that in our experience are reduced inputs unlike other experiences.

CONCLUSION

The treatments (experimental factors) applied on the *Agrostis capillaris* L. - *Festuca rubra* L. grassland, produced weak changes with minimal repercussions on phytodiversity (this explain why the results do not have statistical assurance). However, some species changed their participation, but no one is threatened by extinction from the grassy carpet.

All the treatments tested by us (mulching combined with organic fertilization in different quantities and intervals of time) could become viable solutions of sustainable use of the oligotrophic grassland.

To maintain the oligotrophic grasslands phytodiversity in Apuseni Mountains and slightly increase the dry matter yield, we recommend the variant mulching once a year + 10 t/ha manure, applied once to 2 years (V5), which had highest dry metter yield than the control variant with 0.19 t/ha.

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